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Quantification of solute–solute interactions using negligible-depletion solid phase microextraction: Measuring the affinity of estradiol to bulk organic matter

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Abstract

The interaction of trace organic contaminants with bulk organic matter has implications for the transport and behaviour of organic trace contaminants within the aquatic environment as well as water and wastewater treatment processes. Partition coefficients (K_{OM}) of the steroidal trace organic contaminant estradiol were quantified for environmentally relevant concentrations of bulk organic matter (12.5 mgC/L) using a full mass balance form of solid phase microextraction (SPME). The results indicated that the method is successful and can be used at environmental concentrations. Estradiol had the greatest affinity for bulk organic matter that contained phenolic and benzoic acid ester groups, namely tannic acid, compared to organics containing predominately carboxylic functional groups. The solution chemistry (pH) was found to influence the interaction, as estradiol had a lower affinity for negatively charged and hydrophilic bulk organic matter. The partition coefficients determined using SPME were consistent with partition coefficients derived using solubility enhancement and fluorescence quenching measurements, confirming that SPME is a powerful technique to quantify the affinity of estradiol for low concentrations of bulk organic matter and trace contaminants. Further, this novel method can be applied to a range of trace contaminants.

Introduction

The behaviour and transport of trace organic contaminants within aquatic systems is affected by their affinity for bulk organic matter (*1-3*). This interaction can be described as a 'solute-solute interaction', and has implications for aqueous solubility (*4*), biological uptake and contaminant degradation (*5*) as well as removal of trace organic contaminants in water and wastewater treatment systems (*6*, *7*). However, measuring such interactions at environmentally relevant (generally low) concentrations has, to date, been difficult.

Many trace organic contaminants have adverse effects on environmental and human health. Of particular concern are endocrine disrupting chemicals due to their potential to interfere with the endocrine system leading to effects on reproduction of vertebrates (*8*). Endocrine disrupting

chemicals include natural and synthetic hormones, certain industrial chemicals and pesticides and pharmaceutically active products (*9*). The trace organic contaminant estradiol (E2) was selected as it is a steroidal hormone which is produced naturally by humans and some animals, and it is considered one of the most potent endocrine disrupting chemicals (*10*). The aquatic environment has a greater susceptibility to estradiol and other trace organic contaminants compared to other environments, due to the constant introduction of wastewater effluent (*11*). Effluent from conventional biological treatment plants can have estradiol concentrations ranging from low nanograms per litre to micrograms per litre (*8*) and researchers have demonstrated that concentrations as low as 5 ng/L of estradiol can have significant implications for aquatic life (*12*).

The bioavailability of estradiol within an aquatic system can be influenced by the type of bulk organic matter present (*13*). Bulk organic matter can be used to describe particulate, colloidal and dissolved phases of organic matter of varying origins and characteristics. In water and wastewater bulk organic matter can include natural organic matter (NOM) surrogates, polysaccharides, polyphenols and surfactants. The charge, polarity, aromaticity, hydrophobicity and molecular weight of bulk organic matter can all affect the interaction with trace organic contaminants (*14, 15*). Therefore, as the different types of bulk organic matter have different properties, it is expected that estradiol will show a greater affinity for certain types of bulk organic matter compared to others and hence different transport and treatment properties depending on water composition. Therefore, the purpose of this research is to quantify the interaction of estradiol with a wide range of bulk organic matter at environmentally relevant concentrations. In addition, the interaction will be studied at different pH values (4-9). Estradiol is a weak acid with an acid dissociation constant (pKa) of 10.23, hence it will be mainly undissociated under the studied conditions (6% dissociated at pH 9) (*16*). Most bulk organics have ionisable groups, and therefore partitioning will be influenced by pH (*17*).

Previously, the interaction of contaminants like estradiol with various types of bulk organic matter has been studied using techniques such as fluorescence quenching (FQ) (*18, 19*) and solubility enhancement (SE) (*18*). However, these techniques have limitations which can restrict measurement of partition coefficients, particularly at low concentrations. For example, SE requires a high concentration of bulk organic matter, often in excess of 100 mg/L (approximately 52 mg carbon per litre (mgC/L) for humic acid) (*4*), while in FQ the presence of molecular oxygen can quench trace organics, which overestimates partitioning (*20*). Researchers have previously identified the need for further investigation of this interaction using techniques such as negligible-depletion solid phase microextraction (nd-SPME) (*18*). nd-SPME is based on the principle that if less than 5% of freely dissolved trace organic contaminants are extracted, the extraction does not significantly disturb the equilibrium with bulk organic matter (*21*). nd-SPME has been previously used to quantify the interaction between chlorobenzenes and dissolved organic carbon (*22*), estradiol and protein (bovine serum albumin) (*21*) and DDT and humic acid (*23*).

Materials and Methods

Chemicals. All chemicals were of analytical grade. NaOH and HCl (1 M) were used for pH adjustments and 1 mM $NaHCO_3$ 20 mM NaCl was the background electrolyte (Sigma Aldrich UK). Radiolabelled [2,4,6,7-^{3}H]Estradiol (3.15 TBq/mmol, 37 MBq/mL) was purchased from GE Healthcare UK, while non-labelled 17β-Estradiol (>98% purity) was purchased from Sigma Aldrich UK. Estradiol is a moderately hydrophobic steroidal hormone (log K_{OW} 3.94-4.01) with a solubility of 13 mg/L (in water at 20°C) (*24*). The estradiol concentrations ranged from 100 ng/L to 100 µg/L. This concentration range was selected as it represents realistic concentrations of estrogens in conventional wastewater effluent (*10*).

Bulk Organic Matter. Several different types of organic matter were studied including NOM surrogates, polysaccharides, polyphenols and surfactants. These were selected as representatives of organic matter found in water and wastewater. Suwannee River standard IHSS Humic Acid II (HA)

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(Cat. No. 2S101H), IHSS Fulvic Acid (FA) (Cat. No. 1S101F) and IHSS NOM (Cat. No. 1R101N) were purchased from International Humic Substance Society, USA. Australian NOM was concentrated using microfiltration and reverse osmosis from Brisbane Water National Park, Australia and has been characterised extensively (*25*). Aldrich humic acid (HA) (sodium salt), alginic acid (sodium salt), powder cellulose, colloidal cellulose, tannic acid, and sodium dodecyl sulfate (SDS) (≥96%) were all purchased from Sigma Aldrich, UK. Dextran was purchased from Acros Organics, UK. Aldrich HA may not be an accurate representative of natural terrestrial humic acid (*26*), but was studied as it is a very commonly used material. The concentration of organic matter in natural water can vary greatly, and can range from 0.5 to 100 mgC/L (*27*). A bulk organic matter concentration of 12.5 mgC/L was used in all experiments. Selected characteristics of the bulk organic matter used in this experiment and their origins are given in Table 1. SUVA (specific ultraviolet absorption at 254 nm divided by organic carbon concentration) was also determined for each organic and is an indicator of aromaticity (*28*).

SPME Fibre. Polyacrylate (PA) fibre with a fibre coating thickness of 34.5 µm was purchased from Polymicro Technologies (Phoenix, USA). PA was selected as it is suitable to extract polar compounds, such as estradiol (*29*).

SPME Protocol. Radiolabelled estradiol and the selected bulk organic matter were added to 100 mL pH adjusted buffer solutions containing deionised water (4-9). The solutions were shaken for 24 hours in a Certomat BS-1 incubator shaker, Sartorius (Göttingen, Germany) at 200 RPM and a temperature of 25°C. 5 cm of PA fibre was then added to each solution and shaken for a further 48 hours. This time was selected as preliminary fibre kinetic experiments required to calculate fibre-water partition coefficients indicated equilibrium was reached before 48 hours. Degradation of estradiol during the experiment was assumed to be negligible. Several experiments were repeated with the addition of biocide, 0.5% sodium metabisulfite ($Na_2S_2O_5$), or a darkened setting, but showed no difference in uptake of estradiol by the fibre. After 48 hours the fibres were removed and added to scintillation vials with 7 mL Ultima Gold LLT scintillation fluid (Perkin Elmer, Waltham, USA), and allowed to desorb for 3 hours, after being shaken briefly. The scintillation vials were counted in a Beckman LS 6500 liquid scintillation counter (Fullerton, USA).

Determination of Concentrations with SPME: A relatively low concentration of bulk organic matter (12.5 mgC/L) was used to measure the interaction in natural conditions. As a result the difference between the initial concentration of estradiol in solution (C_{tot}) and the concentration of estradiol freely dissolved at equilibrium (C_W) was small resulting in higher error compared to the method applied to higher concentrations. In addition, the mass of estradiol extracted by the fibre (C_f) was similar to the mass of estradiol partitioning to the bulk organic, and as a result the typical nd-SPME assumptions could not be applied. Therefore, a full mass balance (Equation 1) was required to explicitly account for the mass of estradiol sorbed to the organic matter (C_{OM}). The concentration of estradiol freely dissolved in solution at equilibrium (C_W) was calculated based on the mass of estradiol on the fibre.

$$C_{tot} = C_f + C_W + C_{OM} \qquad \textbf{(1)}$$

Determination of Organic Matter Partition Coefficient (K_{OM}): K_{OM} is used to relate the concentration of estradiol sorbed to the bulk organic matter to the freely dissolved concentration of estradiol in solution (*30*). This is calculated using Equation 2

$$K_{OM} = \frac{C_{OM}}{C_W} \qquad \textbf{(2)}$$

K_{OM} is the bulk organic matter-water partition coefficient (L/kg)
C_{OM} is concentration of estradiol sorbed to the bulk organic matter (ng/kg)
C_W is concentration of estradiol freely dissolved in solution at equilibrium (ng/L)

K_{OM} can be derived from the slope of the linear regression of C_{OM} as a function of C_W if sorption isotherms are linear. Since the concentration range is over several orders of magnitude the sorption isotherms were plotted on a log scale (Equation 3). The slope of the regression, n_i, indicates the deviation from linearity of the sorption isotherm (*3*). Since this value was close to 1 for all isotherms n_i was set to 1, which is equivalent to the linear relationship described by Equation 2.

$$\log C_{OM} = \log K_{OM} + n_i \cdot \log C_W \qquad \textbf{(3)}$$

The random error associated with the quantification of partition coefficients, calculated using error propagation (Equation 4), was 5.4%. %E represents the variability associated with fibre length, as well as errors associated with laboratory equipment such as micropipettes, Hamilton syringes and electronic balances.

$$\%E_{total} = \sqrt{(\%E_1)^2 + (\%E_2)^2 + (\%E_3)^2} \qquad \textbf{(4)}$$

Results and Discussion

Organic Matter Partition Coefficient (K_{OM}). The affinity of estradiol for bulk organic matter was measured as a function of bulk organic matter type (11 organics) and pH (4-9). The sorption isotherms, plotted as the freely dissolved estradiol concentration (ng/L) versus bound estradiol concentration (ng/kg), are shown in Figure 1 A-K. The majority of the isotherms had correlation coefficients greater than 0.95. The log K_{OM} values listed in Table 2. Previously, studies have suggested that an increase in bulk organic concentration can reduce estradiol partitioning (*31*). Therefore, the partition coefficients may be different at higher bulk organic concentrations.

Effect of Bulk Organic Matter (Neutral pH): Properties of the bulk organic matter, such as size and structure, can influence partitioning (*3*). However the interaction of estradiol with bulk organic matter is primarily related to the functional groups present and the molecular interactions between the solute and sorbent. The functional group contents (^{13}C NMR) for NOM surrogates are shown in Table 1, however functional group contents were not available for the other organics used. Based on the characteristics of estradiol and bulk organic matter the mechanisms of interaction are expected to be hydrogen bonding and van der Waals interactions. Hydrogen bonding is a specific dipole-dipole interaction that occurs between hydrogen donors and acceptors. Functional groups that act as both hydrogen donors and acceptors are termed bipolar and include phenol, carboxylic and hydroxyl groups (*32*). Estradiol contains phenol and hydroxyl functional groups therefore it exhibits donor and acceptor properties. In addition, the aromatic rings of estradiol are electron rich and may serve as a weak hydrogen acceptor. The bulk organic matter studied mainly contained bipolar oxygenated functional groups (*3*). The NOM surrogates are composed of a wide range of functional groups, including carboxylic, phenolic and carbonyl groups (*33*) while the polysaccharides mainly contain hydroxyl moieties. Tannic acid primarily contains phenolic, catechol and gallic acid moieties (*34*). SDS is the only bulk organic studied which does not contain a bipolar functional group, however it has a monopolar sulfate moiety.

For all bulk organics the linearity of the sorption isotherms suggests that all sorbents act as partitioning sorbents and adsorption does not play a role (*3*). This linearity is consistent with previous findings for estradiol (*35, 36*).

At pH 7 the bulk organic matter-water partition coefficients were all similar, except for SDS and tannic acid, and ranged from log K_{OM} 4.21 to 3.75 (Table 2). This range corresponds to a factor of

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three of the partition coefficients with the exception of tannic acid and SDS, which were significantly higher (4.86±0.26) and lower (3.68±0.20) respectively.

The bulk organic-water partition coefficients for NOM surrogates ranged from 4.21±0.23 to 3.95±0.21 (Table 2). There was no significant difference in the partition coefficients. It was thought that the origin of the bulk organic would influence partitioning, as this can affect the aromaticity, structure and functional group content. For example, terrestrial humic acids, such as Aldrich HA, are typically more aromatic and contain more phenolic and carboxylic groups compared to aquatic humics, such as IHSS HA (*37*). However, there was no significant difference in partition coefficients for Aldrich HA and IHSS HA (4.21±0.23 and 3.99±0.22 respectively). It should be noted that Aldrich HA was not pre-treated prior to the sorption experiments. Also, Australian NOM was not purified, hence it contained salts common in surface water (*25*). Nevertheless Australian and IHSS NOM had very similar K_{OM} indicating that the presence of salts present in concentrated organic matter does not influence the partitioning behaviour of a neutral compound significantly.

The tannic acid-water partition coefficients (Figure 1 K) were significantly higher than the other interactions, indicating tannic acid was the strongest sorbent (log K_{OM} 4.86 ±0.26). This has been observed previously in other studies with estradiol (*6, 18*) and may be related to the large fraction of phenolic hydroxyl groups in tannic acid. A study by Jin *et al.* (*38*) indicated that phenolic content, as opposed to total aromaticity, determines sorption of hormones with a phenolic moieties, such as estradiol and estrone. In addition at pH 7 any carboxylic acid groups are deprotonated, so tannic acid is the only organic matter in the selection that is neutral and of moderate hydrophobicity (Log K_{OW} 2.2).(*39*)

The bulk organic-water partition coefficients for the polysaccharides, alginic acid, dextran and cellulose, were all similar to the NOM surrogates, despite polysaccharides having lower aromaticity (SUVA values in Table 1). Log K_{OM} ranged from 3.75±0.2 to 3.96±0.21 (Table 2). It has been suggested that bulk organic matter lacking aromatic functional groups, such as dextran, has little effect on the fate and behaviour of steroidal hormones (*38*). However, the similarity in polysaccharide partition coefficients to the NOM surrogates may be due their hydrophilicity, thus increasing the potential for hydrogen bonding. The elemental stoichiometric ratios (specifically (O+N)/C), which are an indirect method to measure polarity (*40*), suggest that the selected polysaccharides are more polar than polyphenol and NOM surrogates (*18, 41*). In addition, all of the polysaccharides contain hydroxyl functional groups, enabling them to act as both a hydrogen donor and acceptor. Therefore, despite the lower aromaticity of these bulk organics, they still interact strongly with estradiol through hydrogen bonding. The large variation in molecular weight of the different polysaccharides (162-210000 g/mol) (Table 1) suggests that molecular weight does not appear to have a significant influence on the interaction of estradiol with polysaccharides.

The SDS-water partition coefficient (Figure 1 J) was significantly lower than all other bulk organics (log K_{OM} 3.68±0.20). SDS is an anionic surfactant, which contains a hydrophilic head, with a hydrophobic tail (*42*). Partitioning is primarily expected to occur through weak hydrogen bonding with the hydrophilic head of SDS, which is not bipolar but only a hydrogen acceptor (sulfate group). In contrast, all other bulk organics are bipolar, thus allowing both hydrogen donating and accepting activities. As the SDS concentration is below the critical micelle concentration (0.0082 M at 25°C) micelle formation is not a factor in partitioning. It is unlikely that the hydrophobic tail of SDS contributes significantly to the interaction, as research by Yamamoto *et al.* (*18*) has indicated that hydrogen bonding is the primary mechanism of interaction between organic matter and estradiol, compared to non-specific interactions.

Effect of solution chemistry (pH): The interaction of estradiol with bulk organic matter was studied from pH 4 to 9. Figure 2 shows a decrease in partition coefficient from neutral to alkaline

pH. With the exception of SDS and tannic acid, this difference was generally less than a factor of 3, and not considered to be significant. A decrease in partition coefficient at alkaline pH has been observed previously when studying the interaction of phenolic compounds with commercial humic acid using SPME (*13, 43*).

It is expected that this pH dependence of partition coefficient is related to the dissociation of bulk organic matter, not the speciation of estradiol. Estradiol is a weak acid (pKa 10.23) and therefore can be affected by pH. However, at pH 9 there is still 94% neutral species compared to almost 100% neutral species from pH 4 to 8. As the dissociation of estradiol is minimal, it is not expected to significantly contribute to a decrease in partitioning. In addition, any changes in fibre characteristics are unlikely to affect partitioning. Zeta potential measurements (mV) of the fibre indicated no change in charge in the studied pH range.

In contrast, most bulk organic matter is affected by pH in the investigated pH range. Phenolic functional groups deprotonate at alkaline pH values (pKa 9.9 of unsubstituted phenol), while carboxylic groups deprotonate under acidic conditions (pKa around 4.5) (*33, 44*). Due to the high carboxylic functional group content in the majority of bulk organic matter studied, most are negatively charged at neutral pH. pH changes can have implications on intramolecular bonding within the bulk organic matter, as well as molecular shape. In acidic solutions NOM surrogates can coil due to intramolecular hydrogen bonding, while at neutral and alkaline pH values the NOM has a linear structure (*45*). The conformational changes in alginic acid are similar, however it depolymerises above pH 8 (*46*). This change in bulk organic matter structure is expected to affect the interaction with estradiol.

Tannic acid is abundant with polyphenol structures (mainly gallic acid) and all carboxylic acid groups are deprotonated. The first pKa of gallic acid is 8.7 (*34*), therefore tannic acid will start to go from neutral to anionic around pH 7 to 8, which explains the large pH dependence of the K_{OM} of one order of magnitude between pH 4 and 9.

Comparison with other quantification techniques: The interaction of estradiol with bulk organic matter has been quantified previously using FQ and SE. Yamamoto *et al.* (*18*) calculated the interaction between estradiol and Suwannee River (IHSS) FA and HA, pre-filtered Aldrich HA and tannic acid using FQ, while the interaction between estradiol and IHSS HA, alginic acid, tannic acid and dextran was quantified using SE. The partition coefficients calculated using SPME were compared to these two methods in Figure 3. SPME, FQ and SE are very different techniques, and therefore have different advantages and limitations. As mentioned previously, SE can be limited by the concentration of bulk organic matter used in the experiments, while FQ partition coefficients are based on assumptions regarding static quenching compared to dynamic quenching (*47*). SPME was selected as it is a simple and sensitive technique which is suitable for low bulk organic concentrations (*21*). However some limitations include susceptibility of fibres to damage (*29*), the possibility of organic fouling and the inability of the polyacrylate fibre to extract charged species.

In Figure 3 the partition coefficients calculated using FQ were a factor of 2.6 to 8.5 times larger than those calculated using SPME. Since concentration of bulk organic matter used in the SPME experiments was in the same concentration range, this can be ruled out as a difference. Consistent with the present observation, previous studies comparing SPME and FQ for different trace organics, such as pyrene, have observed FQ partition coefficients a factor of 5 to 10 times higher than SPME partition coefficients (*48, 49*). Doll *et al*. (*49*) has indicated that this is due to limitations associated with SPME, as it can disturb contaminants weakly bound to the outer shell of bulk organic matter and lead to a higher concentration in the aqueous phase, therefore underestimating partitioning. Partition coefficients calculated using SPME and SE were similar for IHSS HA, tannic acid and alginic acid but significantly different for dextran, despite similar molecular weight fractions used

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in both experiments (Figure 3). The difference between the results is expected to be due to the dextran organic carbon concentration, which was as high as 263 mgC/L.

In conclusion, the SPME method has been shown to work for environmentally relevant concentrations of bulk organic matter and trace contaminants. The results of the present study demonstrate how the interaction of estradiol with bulk organic matter at environmentally relevant concentrations is influenced by the type of bulk organic matter and pH. Partition coefficients presented here can be applied directly to calculate the interaction with organic matter studied here. Further, the method can be applied to determine partition coefficients of other trace organics with any bulk organic matter. Knowledge of bulk organic matter-water partition coefficients are required for environmental models, such as the fugacity model (*50*), in order to understand the fate and behaviour of trace contaminants in the environment. While the typical nd-SPME assumptions were not applicable in experiments with environmentally realistic organic matter concentrations, the technique was suitable if the full mass balance of the system was used. The method is more tedious than typical nd-SPME, but is more generally applicable and versatile, and can in principle be used for a wide range of organic contaminants.

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Table 1

Bulk Organic Type	Molecular Formulae	Category	Charge at Neutral pH	Origin in Water	Carbon %	Carbon Composition % (^{13}C NMR)	Molecular Weight (g/mol)	SUVA (L/mg/m)* (Specific ultraviolet absorption at 254 nm)	Ref
Alginic acid sodium salt	$[C_6H_7NaO_6]_n$	Polysaccharide	⊖	Brown seaweed and algae	36	-	210000	0.019	(*51*)
Powder and colloidal cellulose	$[C_6H_{10}O_5]_n$	Polysaccharide	⊖	Toilet paper, plant decay	44	-	162	0.10 (powder) , 0.37 (colloidal)	(*41*)
Dextran	$[C_6H_{10}O_5]_n$	Polysaccharide	Neutral	Produced by bacteria	44	-	3000-200000	0.02	(*18*, *52*)
IHSS FA	-	NOM surrogate	⊖	Peat and decomposing vegetation	53	Aliphatic: 49 Aromatic: 24 Carboxyl: 20 Carbonyl: 7	1000-2300	3.30	(*25*, *28*, *53*, *54*)
IHSS HA Aldrich HA	-	NOM surrogate	⊖	Peat and decomposing vegetation (IHSS HA) and soil or peat (AHA)	53 -56	Aliphatic: 37 (IHSS) 59 (AHA) Aromatic: 37 (IHSS) 26 (AHA) Carboxyl: 19 (IHSS) 9 (AHA) Carbonyl: 8 (IHSS), 6 (AHA)	600-60000	4.07 (AHA), 4.22 (IHSS HA)	(*25*, *26*, *28*, *53*, *54*)
IHSS NOM Australian NOM†	-	NOM surrogate	⊖	Peat, soil and decomposing vegetation	6.3 - 53	Aliphatic: 49 (IHSS) Aromatic: 23 (IHSS) Carboxyl: 20 (IHSS) Carbonyl: 8 (IHSS)	1381 (Aus NOM)	3.05 (IHSS NOM), 2.58 (AUS NOM)	(*25*, *53*)
SDS	$CH_3(CH_2)_{11}OSO_3Na$	Surfactant	⊖	Detergents and personal care products	48	-	288	0	(*42*)
Tannic Acid	$C_{76}H_{52}O_{46}$	Polyphenol	Neutral	Plants	54	-	1701	4.23	(*18*)

* Measured data; † Salt composition (*25*)

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DOI: 10.1021/es0717313

Table 2

	pH 4 ± measurement uncertainty	**pH 5 ± measurement uncertainty**	**pH 7 ± measurement uncertainty**	**pH 8 ± measurement uncertainty**	**pH 9 ± measurement uncertainty**	**Fluorescence quenching[1] and solubility enhancement[2] log K_{OM} values at pH 7 (*18*)**
SDS	-	-	3.68 ± 0.20	-	2.87 ± 0.15	-
Powder Cellulose	-	-	3.75 ± 0.20	-	3.89 ± 0.21	-
Colloidal Cellulose	-	-	3.94 ± 0.21	-	3.82 ± 0.15	-
Alginic acid	3.88 ± 0.21	-	3.96 ± 0.21	-	3.42 ± 0.18	3.75[2]
Dextran	-	-	3.96 ± 0.21	-	3.77 ± 0.20	2.76[2]
IHSS NOM	4.23 ± 0.23	3.95 ± 0.21	3.95 ± 0.21	3.71±0.21	3.68 ± 0.20	-
Australian NOM	4.16 ± 0.22	3.86 ± 0.21	3.98 ± 0.21	3.99 ± 0.22	3.84 ± 0.21	-
IHSS HA	4.28 ± 0.23	4.09 ± 0.22	3.99 ± 0.22	4.04 ± 0.22	3.84 ± 0.20	4.92[1]; 4.56[2]
IHSS FA	4.24 ± 0.23	3.65 ± 0.20	4.15 ± 0.22	3.78 ± 0.20	-	4.57[1]
Aldrich HA	4.18 ± 0.23	4.26 ± 0.23	4.21 ± 0.23	4.20 ± 0.23	3.95 ± 0.21	4.94[1]
Tannic Acid	5.11 ± 0.28	5.29 ± 0.29	4.86 ± 0.26	4.51 ± 0.24	4.01 ± 0.22	5.28[1]; 4.94[2]

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Figure 1: Estradiol-organic matter sorption isotherms (measured in a background electrolyte containing 1 mM $NaHCO_3$ and 20 mM NaCl) A) Aldrich HA; B) Alginic acid; C) Australian NOM; D) colloidal cellulose; E) powder cellulose; F) dextran; G) IHSS FA; H) IHSS HA; I) IHSS NOM; J) SDS and K) tannic acid.

Figure 2: Estradiol-organic matter partition coefficients (log K_{OM}) for all bulk organics studied as a function of solution pH. The data points at pH 4 in parenthesis are outliers (experimental error that were repeated in duplicate).

Figure 3: Comparison of estradiol-organic matter partition coefficients calculated in this study using SPME with literature fluorescence quenching (FQ) and solubility enhancement (SE) partition coefficients from Yamamoto *et al.* (*18*).

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Figure 1

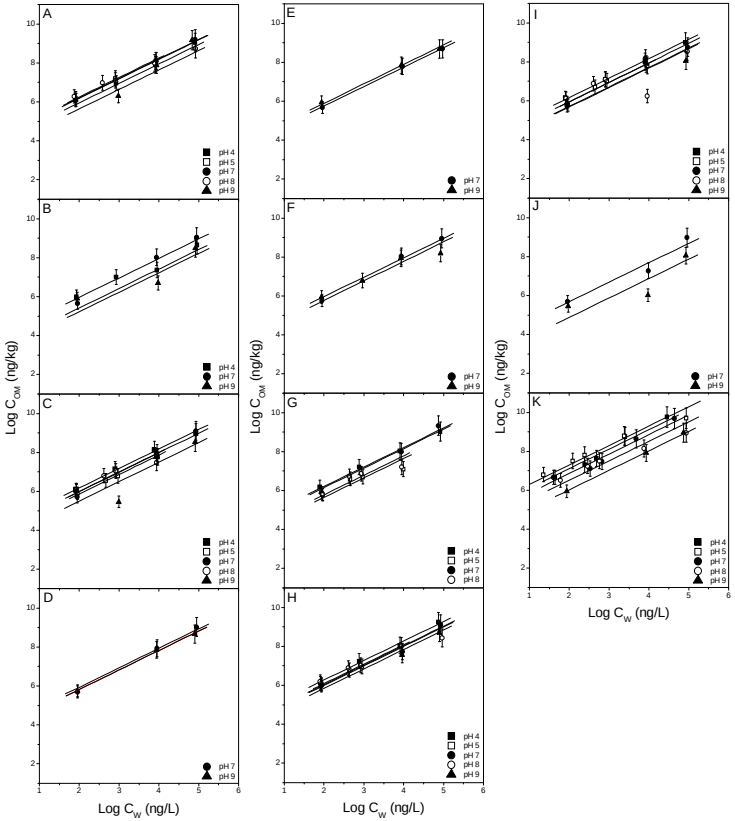

Figure 2

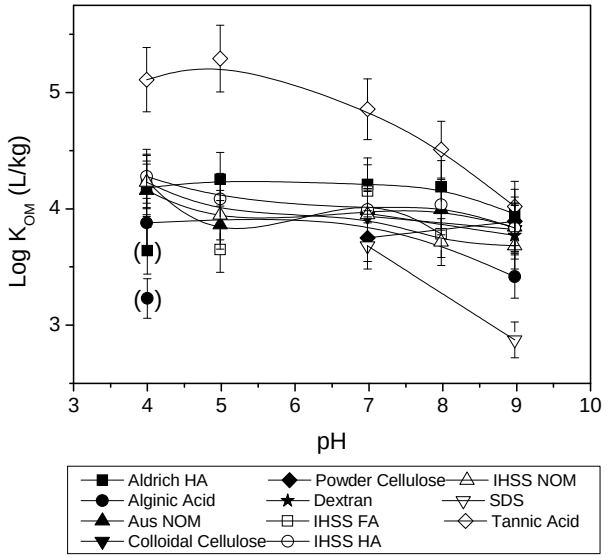

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Figure 3